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ORIGINAL ARTICLE

Invasive mealybug *Pseudococcus jackbeardsleyi* Gimpel and Miller on *Curcuma aeruginosa* Roxb. on the seed rhizome in storage: a record of a new host

Rohimatun[1], Nida Nur Rahmah[2], Dewi Sartiami[2], Mahindra Dewi Nur Aisyah[3], Miftakhurohmah[1]*, Melati[1], Devi Rusmin[1], Tri Lestari Madiningsih[1]

[1] Research Center for Estate Crops, National Research and Innovation Agency (Badan Riset dan Inovasi Nasional – BRIN), Cibinong, Indonesia

[2] Department of Plant Protection, Faculty of Agriculture, IPB University, Bogor, Indonesia

[3] Department of Plant Production, Politeknik Negeri Jember, Jember, Indonesia

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*Corresponding address:
miftahtia05@gmail.com

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Abstract

Mealybug is a renowned pest known to attack agricultural products from the field to the post-harvest process, such as on the seed rhizomes of *Curcuma aeruginosa*. Therefore, this study aimed to examine and identify the species of mealybug on the seed rhizomes of *C. aeruginosa* based on morphological and molecular characteristics. Fifty mealybugs were collected from the seed rhizomes of *C. aeruginosa* in the storage room in Bogor (Indonesia) using a soft brush. They were transferred to new *C. aeruginosa* rhizomes without any other insects present. Morphological identification based on observation of mounted specimens of 10 female adults and six for molecular identification. The primer pair that amplified the mitochondrial cytochrome oxidase I (COI) gene was used to study the molecular characteristics and was continued with direct sequencing and sequence analysis. The results showed that the morphological characteristics of the mounted specimen were close to those of *Pseudococcus jackbeardsleyi*. Amplification of the COI gene yielded DNA bands measuring 490 base pairs (bp), while homology and phylogeny analysis confirmed the morphological identification. Based on BLAST analysis, the similarity of COI genes of mealybugs in this study was above 99% with other *P. jackbeardsleyi*. The study specimen was identified as *P. jackbeardsleyi* on the seed rhizomes of *C. aeruginosa* by comparing the morphological features of insect specimens and results of the species available in GeneBank. This result represented the first documented report about the presence of the species in storage.

Keywords: blue ginger, COI genes, identification, Jack Beardsley, morphological characteristics

Introduction

Blue ginger (*Curcuma aeruginosa*) is an aromatic and herbaceous perennial tuber crop belonging to the Zingiberaceae family. Despite being underexplored, it is highly regarded for its edible and medicinal rhizomes. Its distinguishing features include rhizomes with pink tips and grayish blue or blue centers, as well as leaves adorned with purple or reddish-brown patches along the sides of the midrib on the upper side of the lamina, which fade with maturity. Native to Myanmar, it is extensively cultivated in Malaysia and commonly found throughout southeast Asia (Soorya *et al.* 2018). The rhizomes contain secondary metabolites, such as sesquiterpenes, monoterpenes, phenols, diterpenes, phenanthrene, tetrapeptides, oxazole, triazine,

piperidines, and oxygenated hydrocarbons (Nurcholis *et al.* 2021). This part has been used as a traditional medicine for gastrointestinal problems, uterine pain, postpartum uterine, perimenopausal hemorrhage, and dengue fever (Kamazeri *et al.* 2012). It is also anticancer (Nurcholis *et al.* 2021), and antimicrobial (Aziz *et al.* 2021).

Curcuma aeruginosa has not been widely cultivated in Indonesia, limiting access to the rhizomes as a raw material for herbal medicine. This shortage is due to the reliance on small-scale cultivation in yards, leading to variations in the quality. Cultivation on a comprehensive and intensive scale is needed to obtain quality and standardized raw materials for herbal medicine. This plant is propagated vegetatively using rhizomes hence, an appropriate storage method is needed to meet the demand for the seed.

The long-term storage of *C. aeruginosa* seed rhizomes is often plagued by plant pests, leading to shrinking and decreased viability. Damage caused by insects during storage includes weight loss, reduced water content and quality, discoloration, decreased growth ability, and protein content in embryos as an energy source. Based on the results of previous observations, the insect pests that attack the seed rhizome of *C. aeruginosa* belong to the order Hemiptera and family Pseudococcidae. The rhizomes of the genus *Curcuma* can host insect pests, including *Formicococcus polysperes* Williams (Firake *et al.* 2018) and *Paracoccus marginatus* Williams and Granara de Willink (Chellappan *et al.* 2013). Infected rhizomes during storage can spread the pests through seed distribution between islands and countries. This must be prevented through effective practices, including observation and identification of mealybugs during storage. Initial treatment information can be obtained to reduce attacks and prevent spread to other areas through observation and identification of biological, anatomical, and molecular characteristics.

Morphological identification of certain mealybug species presents a challenge, particularly in identifying nymphs and males, which requires high expertise and time-consuming efforts (Beltrà *et al.* 2012). Molecular markers can be used as a complement to morphological identification, which provides a faster and more accurate identification technique. DNA barcoding, a taxonomic system based on information from short nuclear DNA sequences, has been widely used for the molecular identification of several insect families (Banta *et al.* 2016). The mitochondrial cytochrome oxidase I (COI) gene is the region selected as the standard molecular marker for animal identification. This region has been reported to discriminate between Pseudococcidae species and others, such as Aphididae species (Banta *et al.* 2016; Wieczorek *et al.* 2024).

Information regarding the presence of mealybugs on the *C. aeruginosa* seed rhizomes during storage is limited. Therefore, their existence needs to be explored morphologically and molecularly. This study aimed to examine and identify mealybug species on the seed rhizomes of *C. aeruginosa* from Bogor, West Java, Indonesia, based on morphological and molecular characteristics.

Materials and Methods

This study was conducted in the Insect Pest Bioecology Laboratory and the Molecular Laboratory of the Plant Protection Research Group, the Indonesian for Spice and Medicinal Crops Research Institute (ISMCRI), as well as the Laboratory of Insect Biosystematics at the IPB University of Bogor. The research stages included collection, mass rearing, morphological observation, and molecular characteristics analysis for the mealybug.

Mealybugs collection

Mealybugs on the seed rhizomes of *C. aeruginosa* (Fig. 1) were collected in the storage room of the Seed Production Unit of ISMCRI (Bogor, Indonesia)

Fig. 1. Mealybugs on the seed rhizomes of *Curcuma aeruginosa*

(6°34'44"S–106°47'10"E) at room temperature with a soft brush. No other crops were stored in the storage room. The seed rhizomes of *C. aeruginosa* infested with mealybugs were taken and then placed in a jar plastic covered with gauze for air circulation. Furthermore, 50 mealybugs were transferred to new *C. aeruginosa* rhizomes without any other insects present to be reared and identified.

Mealybug mass rearing

After being collected, the mealybugs were reared on *C. aeruginosa* rhizomes under laboratory conditions (25 ± 2°C, 70 ± 10% relative humidity). The rearing method was modified from González-Hernández *et al.* (2005).

Observation of mealybug morphology

The mass-reared mealybugs were identified to confirm the species, then placed in a Syracuse watch glass containing 6–8 ml of Essig's liquid, and one drop of chloroform was added. A mealybug was pricked on the dorsal submarginal surface and 1–2 drops of fuchsine acid were added. The samples were heated at 60–70°C for 15–30 min followed by cooling. The interior was cleaned by pressing gently with a brush, then the samples were transferred to another Syracuse watch glass containing Essig's liquid and chloroform. Cleaning was further performed until the interior looked transparent. The mealybug was then transferred to a glass slide that had been dripped with Heinz mounting media, then covered with a cover glass and dried at 60°C for 5 min. Mealybug specimens were made from 10 female adults which have a complete morphological structure. Identification keys are available for several genera or complex species (Gullan 2000). Observation and identification of mealybug morphology was done using a compound and stereo microscope (Olympus CX 21FS1) connected to digital camera Leica M250 C. Identification was carried out based on the identification key book by Williams and de Willink (1992) and Williams (2004).

Molecular identification

Six mealybugs were used for molecular identification. Isolation of single nymph or adult DNA referred to the protocol of Banta *et al.* (2016), with the procedure as follows: an Eppendorf tube filled with one mealybug plus a CTAB buffer solution [1% w/v CTAB; 1 M NaCl; 100 mM Tris HCl (pH 8.0); 20 mM EDTA (pH 8.0); 1% w/v PVP], reaching 100 µl. The mealybugs were crushed using a sterile gun, then the solution was incubated at 65°C for 45 min and cooled to room temperature. Subsequently, chloroform : isoamyl alcohol (C : I = 24 : 1= v/v) was added to the same volume (v/v) and mixed by vortexing for about 10 s. The solution was centrifuged at 12,000 rpm for 5 min at room temperature then the top layer was taken carefully and transferred to a new Eppendorf tube. The solution was precipitated by adding 1/10 volume of sodium acetate solution (3 M; pH 5.2) and the same volume of cold isopropanol. The Eppendorf tube was inverted until the solution was mixed thoroughly or a DNA pellet was formed, followed by storage at –20°C in a freezer for 30 min to get more DNA pellets. The solution was centrifuged at 12,000 rpm for 15 min at 4°C, then it was discarded, and the DNA pellet was washed with 70% ethanol two times and air dried at room temperature. To the DNA pellet 20–30 µl of nuclease-free water was added and frozen at –20°C until ready to use. Total DNA quality was measured with the NanoPhotometer Implen (Germany).

DNA amplification used primers targeting the larval cuticle organ (LCO) region of the insect COI gene, namely primers LCO-M-2d-F, ATAACTATACCTATYATTATTGGAAG, and LCO-M-2d-R AATAAATGTTGATATAAAATTGG measuring around 490 bp (base pairs) (Malausa *et al.* 2011). The LCO region of three Indonesian mealybugs from *Piper nigrum* L. was also successfully amplified by these primers in a previous study (Miftakhurohmah *et al.* 2022). The PCR reagent used was 2X MyTaq HS Red Mix (Bioline, Germany) forward and reverse primers (10 µM) 1 µl each, with 1 µl DNA template (about 50–600 µg · µl^{-1}). Nuclease-free water was added until the reagent volume reached 20 µl. The PCR program used was predenaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 1 min, annealing at 48°C for 15 s, extension at 72°C for 1 min, and ending with a final extension at 72°C for 10 min (Malausa *et al.* 2011). For the template of negative control of PCR, nuclease-free water was used. DNA visualization was performed on 1.5% agarose gel in 0.5X TAE buffer using RedSafeTM gel dye (Intron Biotechnology). Gels were observed and documented using a GelDoc fire reader V4 (Uvitec Cambridge).

Confirmation of the amplified PCR product with the primer LCO-M-2d-F/R was carried out by direct sequencing. An initial analysis of the sequence results was conducted using the BLAST program. The corresponding nucleotide sequences were then edited, aligned, and homology analyzed with several related sequences from GenBank using the Bioedit sequence alignment editor program. Phylogenetic tree construction was carried out using the Mega X program (Hall 2013), using the neighbor-joining method with 1,000 bootstrap replications to estimate the evolutionary distance between all sequences simultaneously.

Results

Morphological identification of mealybugs

The sample identification results showed that the mealybug found on the seed rhizomes of *C. aeruginosa* belonged to the species *Pseudococcus jackbeardsleyi* Gimpel and Miller (Hemiptera: Pseudococcidae). The Jack Beardsley mealybug in this study was confirmed by referring to the main external characteristics, such as the bodies of adult females being broadly oval in shape with well-developed legs (Fig. 2A), eight-segmented antennae (Fig. 2B), six discoidal pores on the

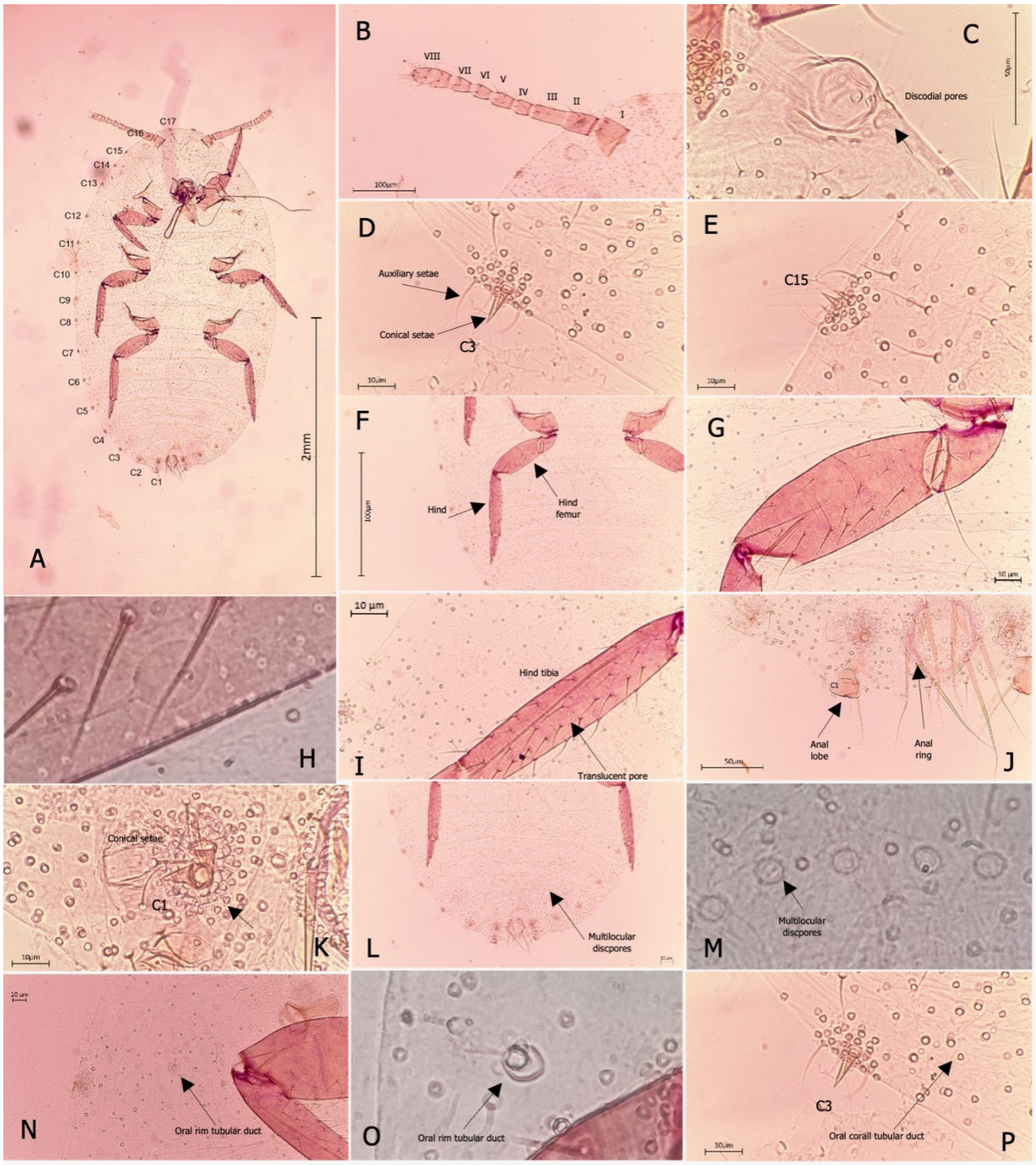

Fig. 2. Characteristic morphology of *Pseudococcus jackbeardsleyi* adult females on the *Curcuma aeruginosa* seed rhizomes. (A) The body is oval and has 17 pairs of cerarii around it. (B) eight segments antenna. (C) Six discoidal pores in the sclerotized area around the eye. (D) Two conical setae on each serarii. (E) Three conical setae on the cerarii of the head. (F) Hind leg. (G) Translucent pores in the hind femur. (H) Translucent pores. (I) Translucent pores in the hind tibia. (J) The sclerotization of the anal lobe is more minor than on the anal ring. (K) Anal lobe cerarii, each containing two stout conical setae and crowded trilocular pores. (L–M) Multilocular disc pores spread on V–VII abdominal segments. (N) Oral rim tubular ducts are present on the thorax. (O) Oral rim tubular ducts have one short setae. (P) Oral collar tubular ducts are located near the cerarii

sclerotized area around the eye (Fig. 2C), 17 pairs of cerarii around the body (Fig. 2A) and each cerarii had two conical setae (Fig. 2D). Cerarii on the head (C15–C17) bear 3–5 conical setae (Fig. 2E) and C7 usually has three conical setae. Translucent pores are on the hind femur and tibia (Figs 2G–I, 2H). Another feature is anal lobe cerarii, each containing two stout conical setae and crowded trilocular pores (Fig. 2K). The sclerotization of the anal lobe is triangular or square and smaller than the anal ring (Fig. 2J). Multilocular disc pores are distributed on V–VII abdominal segments (Fig. 2L–M). Oral rim tubular ducts often have one short seta (Fig. 2O) and one discoidal pore adjacent to the rim. These oral rim tubular ducts are present submarginally on the thorax (Fig. 2N–O), usually submedially in the abdomen and midline. In addition, oral collar tubular ducts are located near the cerarii (Fig. 2P).

Molecular identification of mealybugs

Total DNA amplification of the mealybugs using the primers LCO-M-2d-F and LCO-M-2d-R resulted in DNA bands measuring approximately 500 base pairs (bp). Homology analysis (confirmed with BLAST analysis) showed that the sequences obtained had an identity percentage above 99% with *P. jackbeardsleyi* from various hosts and countries. Approximately 89.50–91.40% resemblance was recorded with other species of mealybugs (*Pseudococcus comstocki, Pseudococcus cryptus, Pseudococcus odermatti, Pseudococcus viburni, Paracoccus gillinae, Atrococcus paludinus, Ferrisia virgata*, and *Planococcus lilacinus* (Table 1).

The LCO sequence of *P. jackbeardsleyi* from *C. aeruginosa* has been registered at GenBank with the accession code LC722456. In the phylogenetic tree, *P. jackbeardsleyi* from Indonesia was close to *P. jackbeardsleyi* from China and grouped with *P. jackbeardsleyi* from North Korea and India, separated from other *Pseudococcus* species (*P. viburni*, *P. comstoki*, *P. odermatti,* and *P. cryptus*), as well as other species of mealybugs *(F. virgata, Pl. lilacinus*, *A. paludinus*, and *Pl. gillinae*). *Aphis fabae* from Italy, which was used as an outgroup, formed a group separate from the mealybug sequence (Fig. 3).

Discussion

The mealybug *P. jackbeardsleyi*, also known as the Jack Beardsley mealybug, originates from the Neotropics and has spread throughout the Americas, as well as portions of Asia, Africa, and Oceania (Wang *et al.* 2018). Initially, this mealybug was unintentionally detected in Hawaii in 1959, then found in Kiribati (1972), Tuvalu (1976), and Papua New Guinea (1979) (Gimpel and Miller 1996). Meanwhile, in Asia, *P. jackbeardsleyi*

Table 1. Nucleotide homology of Indonesian *Pseudococcus jackbeardsleyi* from the *Curcuma aeruginosa* seed rhizome with species of mealybug from GenBank

No	Species	Country	GenBank accession	% similarity
1.	*Pseudococcus jackbeardsleyi*	China	KU296283	99.50
2.	*Pseudococcus jackbeardsleyi*	China	MN901464	99.50
3.	*Pseudococcus jackbeardsleyi*	India	KC119455	99.50
4.	*Pseudococcus jackbeardsleyi*	South Korea	HQ179902	99.30
5.	*Pseudococcus comstocki*	China	KY373167	91.40
6.	*Pseudococcus comstocki*	China	KP692668	91.10
7.	*Pseudococcus cryptus*	China	KP692676	90.10
8.	*Pseudococcus cryptus*	South Korea	HM474373	89.50
9.	*Pseudococcus odermatti*	China	KP692683	91.10
10.	*Pseudococcus odermatti*	China	KP692687	91.10
11.	*Pseudococcus viburni*	China	KP692723	90.10
12.	*Pseudococcus viburni*	France	JQ085549	90.10
13.	*Paracoccus gillinae*	China	KY372944	91.40
14.	*Paracoccus gillinae*	USA	KJ830765	91.40
15.	*Atrococcus paludinus*	China	KY372816	90.70
16.	*Atrococcus paludinus*	South Korea	HM474084	90.70
17.	*Ferrisia virgata*	China	KY373080	90.10
18.	*Ferrisia virgata*	Indonesia	LC596435	90.10
19.	*Planococcus lilacinus*	South Korea	HM474309	90.10
20.	*Planococcus lilacinus*	China	KY373168	89.90

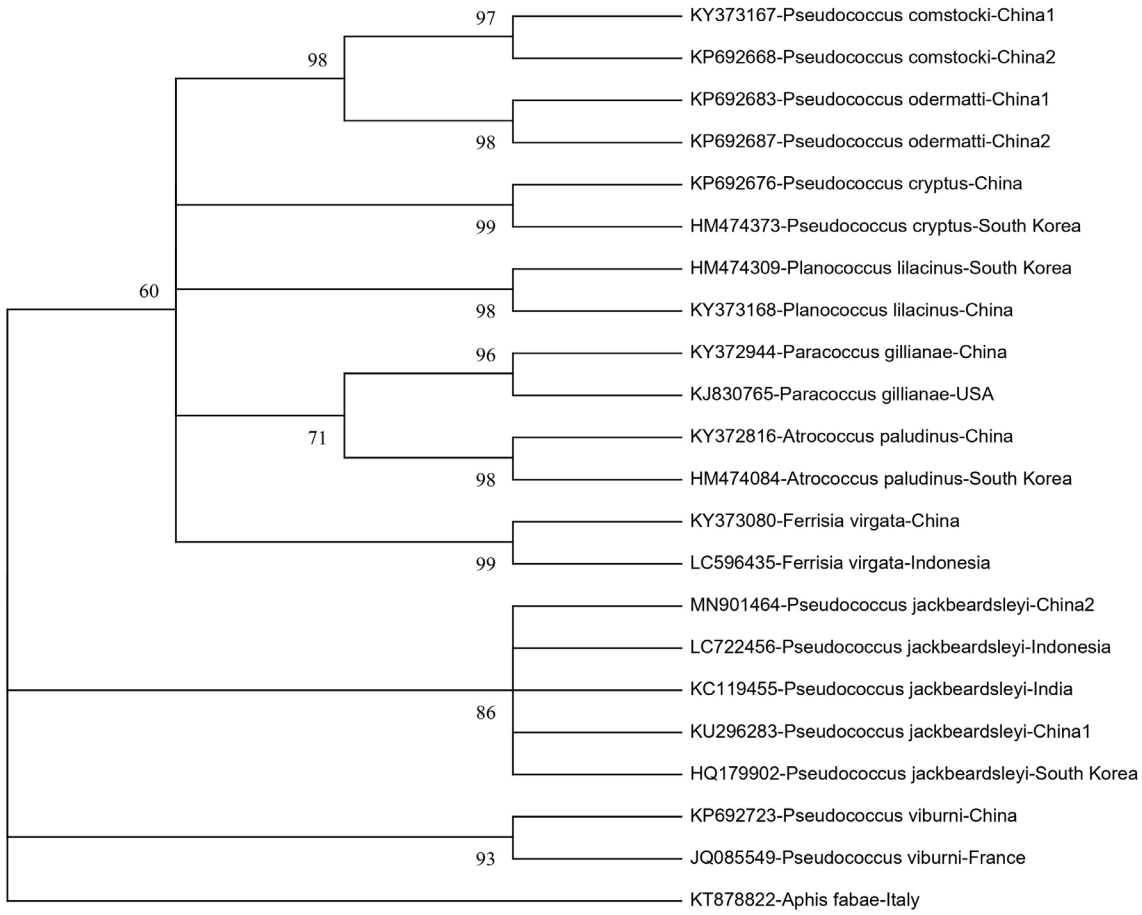

Fig. 3. The phylogeny tree of *Pseudococcus jackbeardsleyi* from *C. aeruginosa* seed rhizomes from Bogor-West Java with *P. jackbeardsleyi* and other species of mealybugs from GenBank. *Aphis fabae* was used as an outgroup

was first reported in Singapore (1958) followed by Malaysia (1969), Indonesia (1973), the Philippines (1975), Brunei Darussalam (1979), Thailand (1987), Maldives, and Vietnam (1994) (Williams 2004).

Pseudococcus jackbeardsleyi mealybugs are small (an adult female measures 3.1 × 1.6 mm, while the newly emerged nymph is 0.7 × 0.4 mm, and the egg 0.5 × 0.3 mm) (Gimpel and Miller 1996). This invasive insect can be easily transported with agricultural products (Miller *et al.* 2002; Muniappan *et al.* 2009). *Pseudococcus jackbeardsleyi* could invade 88 genera in 38 families and 93 plant species (Muniappan *et al.* 2009; CABI 2018). The association between *P. jackbeardsleyi* and their potential plant hosts is poorly studied, especially in Indonesia. Previous records of host plants reported in Indonesia, include *P. nigrum* (Sartiami *et al.* 2010), *Manihot esculenta* (Lena and Puu 2018), and dragon fruit (Sartiami *et al.* 2020). Other host plants include tropical and subtropical fruits, namely pineapple, litchi, mango, banana, durian, dragon fruit, citrus fruit, waxapple, melon, mangosteen, and rambutan, beverage plants, like coffee and cocoa, ornamentals like irises and Chinese roses, vegetables like pepper, tomato, potato, pumpkin, and celery, fiber plants, like cotton, and more (Gimpel and Miller 1996; Mani *et al.* 2013; Palma-Jiménez and Blanco-Meneses 2016; CABI 2018; Wang *et al.* 2018).

In the present study, for the first time *P. jackbeardsleyi* was found attacking on the seed rhizomes of *C. aeruginosa* in storage from *P. nigrum* plantations. In 2005, Sartiami *et al.* (2010) found *P. jackberasleyi* on *P. nigrum* grown in Sukamulya (West Java, Indonesia) (6°56' S 106°46' E) managed by the same institution, providing additional host information. In India (2012), *P. jackbeardsleyi* was reported to be associated with *P. marginatus* attacking papaya plants, potentially causing a reduction in production quality and even crop failure (Mani *et al.* 2013). Therefore, the presence of *P. jackbeardsleyi* must be a concern as it directly reduces seed quality.

Morphological analysis showed the characteristics of *P. jackbeardsleyi,* specifically the adult females. Although Sartiami *et al.* (2010) did not carry out molecular identification, their study reported that the morphological characteristics identified were identical to those of the *P. jackbeardsleyi* species found in *C. aeruginosa*. The presence of 16–17 pairs of cerarii with auxiliary setae on adult females served as one of

the most accessible distinguishing traits (Downie and Gullan 2004).

The physical characteristics of *Pseudococcus* species are highly ambiguous due to the resemblance in the systematics (Gimpel and Miller 1996). *Pseudococcus jackbeardsleyi* and *Pseudococcus elisae* Borchsenius (Hemiptera: Pseudococcidae) (CABI 2018) have similar physical traits, including 17 pairs of cerarii on the body's edge. The anal lobe of the cerarii has two cone-shaped setae, while the C17 cerarii has three. The antenna has eight segments and translucent pores are typically limited to the femur and tibial metacoxas. Moreover, circulus exists between segments III and IV, without an anal bar. There are two oral rim tubular canals in the head next to C17 and approximately nine disc-shaped pores at the edge of the eye. The primary distinction is that *P. jackbeardsleyi* has an oral rim tubular duct in segment VII of the sternal abdomen. Additionally, it has a range of 14–27 oral rim tubular ducts in the tergal abdomen with a maximum of 14 (Gimpel and Miller 1996).

The COI gene of the mealybug from blue ginger had higher similarity with *P. jackbeardsleyi* from several countries (Table 1). Phylogenetic analysis also supported the homology analysis results by grouping the mealybug from *C. aeruginosa* into the *P. jackbeardsleyi* group. Molecular analysis confirmed the morphological identification.

Although *P. jackbeardsleyi* has never been reported as a severe pest, this species has become distributed relatively rapidly and is well established in 47 countries or regions (CABI 2018). This situation could be injurious, specifically due to the absence of suitable natural enemies (Williams and Watson 1988; CABI 2018).

The presence of mealybugs caused *C. aeruginosa* rhizomes to shrivel and become smaller. Early detection of the presence of this pest is important to avoid outbreaks and to know how to manage it (Wang *et al.* 2018). Mani and Shivaraju (2016) stated that mealybugs secrete honeydew in all developmental stages, from early instar nymphs to adults. This secreted material sticks to plant parts, attracting fungus that causes sooty mildew disease capable of reducing the quality of agricultural products. Sooty mold has never been observed on *C. aeruginosa* rhizomes in the field. Additionally, the discovery of mealybugs occurred in storage only recently, and its field-related disadvantages remain unstudied. A notable immediate drawback during storage manifests as shriveled rhizomes leading to weight loss, consequently impacting rhizome quality. In Zingiberaceae cultivation, include *C. aeruginosa*, diseases such as soft rot, anthracnose, and infestations by thrips and scale insects significantly diminish yields. If these pests are detected on the *C. aeruginosa* seed rhizomes in storage facilities, they are likely to transfer to the field, facilitated by other insects, such as ants (Hui and Liu 2023). Therefore, the presence of mealybugs on the *C. aeruginosa* rhizomes necessitates comprehensive management strategies in storage areas and the field to mitigate potential risks. Furthermore, the presence of mealybugs associated with ants (Hymenoptera: Formicidae) can reduce the population of natural enemies, such as parasitoids and other predators. In this case, the ants protect mealybugs so that the control will be constrained (Fanani *et al.* 2020).

It is essential to have a fast and accurate way to identify this invasive pest for plant management, quarantine, and monitoring. The prevention strategy for controlling the spread of the mealybug *P. jackbeardsleyi* in Indonesia generally involves quarantine measures implemented at Pre Border, At Border, and Post Border stages. Control practices are guided by national laws, specifically Law No. 12 (1992) concerning the plant protection system, and Law No. 21 (2019) concerning animal, fish, and plant quarantine. Additionally, these practices adhere to international standards governing global plant protection and health. From this research, it is hoped that further research regarding a comparison of the chemical composition of infested and healthy *C. aeruginosa* seed rhizomes will be done since it is critical to determine direct losses. Timely monitoring and exploration of control techniques management for *P. jackbeardsleyi* will also be necessary in the future so that the attack level during storage can be reduced and spreading via seed rhizomes to other areas can be prevented early.

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References

Aziz J.A., Saidi N.B., Ridzuan R., Mohammed A.K.S., Aziz M.A., Kadir M.A., Abdullah N.A.P., Hussein S., Yusoff H. 2021. Chemical profiling of *Curcuma aeruginosa* Roxb. essential oils and their antimicrobial activity against pathogenic microbes. Journal of Essential Oil-Bearing Plants 24 (5): 1059–1071. DOI: https://doi.org/10.1080/0972060X.2021.1971570

Banta G., Jindal V., Mohindru B., Sharma S., Kaur J., Gupta V.K. 2016. Molecular phylogenetic analysis of mango mealybug, *Drosicha mangiferae* from Punjab. Journal of Environmental Biology 37 (1): 49–55. Available on: http://www.jeb.co.in/journal_issues/201601_jan16/paper_08.pdf [Accesed: 3 July 2023]

Beltrà A., Soto A., Malausa T. 2012. Molecular and morphological characterization of Pseudococcidae surveyed on crops

and ornamental plants in Spain. Bulletin of Entomological Research 102 (2): 165–172. DOI: https://doi.org/10.1017/s0007485311000514

CABI (CAB International). 2018. PlantwisePlus Knowledge Bank: *Pseudococcus jackbeardsleyi* (Jack Beardsley mealybug). Available on: https://plantwiseplusknowledgebank.org/doi/10.1079/PWKB.Species.45087 [Accessed: 4 July 2023]

Chellappan M., Lawrence L., Indhu P., Cherian T., Anitha S., Jimcymaria T. 2013. Host range and distribution pattern of papaya mealy bug, *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) on selected Euphorbiaceae hosts in Kerala. Journal of Tropical Agriculture 51 (1–2): 51–59. Available on: https://jtropag.kau.in/index.php/ojs2/article/view/281/281 [Accessed: 3 July 2023]

Downie D.A., Gullan P.J. 2004. Phylogenetic analysis of mealybugs (Hemiptera: Cocoidea: Pseudococcidae) based on DNA sequences from three nuclear genes, and a review of the higher classification. Systematic Entomology 29: 238–259. DOI: https://doi.org/10.1111/j.0307-6970.2004.00241.x

Fanani M.Z., Rauf A., Maryana N., Nurmansyah A., Hindayana D. 2020. Parasitism distrupsion by ants of *Anagyrus lopezi* (Hymenoptera: Encyrtidae), parasitoid of cassava mealybug. Biodiversitas 21 (6): 2337–2353. DOI: https://doi.org/10.13057/biodiv/d210601

Firake D.M., Joshi S., Rajappa J.J., Behere G.T. 2018. Invasive hypogeic mealybug, *Formicococcus polysperes* Williams (Hemiptera: Pseudococcidae) on turmeric (*Curcuma longa* Linnaeus): a new host record. National Academic Science Letter 41 (6): 341–343. DOI: https://doi.org/10.1007/S40009-018-0692-4

Gimpel W.F., Miller D.R. 1996. Systematic analysis of the mealybugs in the *Pseudococcus maritimus* complex (Homoptera: Pseudococcidae). Contributions on Entomology, International 2 (1): 1–163. Available on: https://www.researchgate.net/publication/284697261_Systematic_analysis_of_the_mealybugs_in_the_Pseudococcus_maritimus_complex_Homoptera_Pseudococcidae#fullTextFileContent [Accessed: 29 June 2023]

González-Hernández H., Pandey R.R., Johnson M.W. 2005. Biological characteristics of adult *Anagyrus ananatis* Gahan (Hymenoptera: Encyrtidae), a parasitoid of *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae). Biological Control 35 (2): 93–103. DOI: http://dx.doi.org/10.1016/j.biocontrol.2005.07.014

Gullan P.J. 2000. Identification of the immature instars of mealybugs (Hemiptera: Pseudococcidae) found on citrus in Australia. Australian Journal of Entomology 39 (3): 160–166. DOI: https://doi.org/10.1046/j.1440-6055.2000.00171.x

Hall B.G. 2013. Building phylogenetic trees from molecular data with MEGA. Molecular Biology and Evolution 30 (5): 1229–1235. DOI: https://doi.org/10.1093/molbev/mst012

Hui J., Liu N. 2023. Main pests and diseases of Zingiberaceae and their control. American Journal of Plant Sciences 14 (9): 988–993. DOI: https://doi.org/10.4236/ajps.2023.149067

Kamazeri T.S.A.T., Samah O.A., Taher M., Susanti D., Qaralleh H. 2012. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. Asian Pacific Journal of Tropical Medicine 5 (3): 202–209. DOI: https://doi.org/10.1016/S1995-7645%2812%2960025-X

Law No. 12. 1992. Sustainable plant cultivation system. Available on: https://peraturan.bpk.go.id/Details/46604/uu-no-12-tahun-1992 (in Indonesia) [Accessed: 24 March 2024]

Law No. 21. 2019. Animal, Fish, and Plant Quarantine. Available on: https://peraturan.bpk.go.id/Details/123687/uu-no-21-tahun-2019 (in Indonesia) [Accessed: 24 March 2024]

Lena W., Puu Y.M.S.W. 2018. Diversity of mealybug types pests on cassava plants in Ende City. Agrica 11 (1): 51–59 (in Indonesia). DOI: https://doi.org/10.37478/agr.v11i1.22

Malausa T., Fenis A., Warot S., Germain J.F., Ris N., Prado E., Botton M., Vanlerberghe-Masutti F., Sforza R., Cruaud C., Couloux A., Kreiter P. 2011. DNA markers to disentangle complexes of cryptic taxa in mealybugs (Hemiptera: Pseudococcidae) Journal of Applied Entomology 135 (1–2): 142–155. DOI: https://doi.org/10.1111/j.1439-0418.2009.01495.x

Mani M., Joshi S., Kalyanasundaram M., Shivaraju C., Krishnamoorthy A., Asokan R., Rebijith K.B. 2013. A new invasive jack beardsleyi mealybug, *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) on papaya in India. Florida Entomologist 96 (1): 242–245. DOI: http://dx.doi.org/10.1653/024.096.0135

Mani M., Shivaraju C. 2016. Biology in mealybugs and their management in agricultural and horticultural crops. p. 67–106. In: "Mealybugs and Their Management in Agricultural and Horticultural Crops" (M. Mani, C. Shivaraju, eds.) Springer, New Delhi.

Miftakhurohmah, Hidayat S.H., Mutaqin K.H., Soekarno B.P.W., Wahyuno D. 2022. Identification of mealybugs on *Piper nigrum* as vector of piper yellow mottle virus (Badnavirus: Caulimoviridae). Journal of Tropical Plant Pests and Diseses 22 (2): 144–153. DOI: https://doi.org/10.23960/jhptt.222144-153

Miller D.R., Miller G.L., Watson G.W. 2002. Invasive species of mealybugs (Hemiptera: Pseudococcidae) and their threat to U.S. agriculture. Proceedings of the Entomological Society of Washington 104 (4): 825–836. Available on: https://www.researchgate.net/publication/297354423_Invasive_species_of_mealybugs_Hemiptera_Pseudococcidae_and_their_threat_to_US_agriculture#fullTextFileContent [Accessed: 28 June 2023]

Muniappan R., Shepard B.M., Watson G.W., Carner G.R., Rauf A., Sartiami D., Hidayat P., Afun J.V.K., Goergen G., Rahman A.K.M.Z. 2009. New records of invasive insects (Hemiptera: Sternorrhyncha) in Southeast Asia and West Africa. Journal of Agricultural and Urban Entomology 26 (4): 167–174. DOI: https://doi.org/10.3954/1523-5475-26.4.167

Nurcholis W., Khumaida N., Bintang M., Syukur M. 2021. GC-MS analysis of rhizome ethanol extracts from *Curcuma aeruginosa* accessions and their efficiency activities as anticancer agent. Biodiversitas 22 (3): 1179–1186. DOI: https://doi.org/10.13057/BIODIV%2FD220313

Palma-Jiménez M., Blanco-Meneses M. 2016. First record of morphological and molecular identification of mealybug *Pseudococcus jackbeardsleyi* (Hemiptera: Pseudococcidae) in Costa Rica. Universal Journal of Agricultural Research 4: 125–133. DOI: https://doi.org/10.13189/UJAR.2016.040403

Sartiami D., Saptayanti N., Syahputra E., Mardiasih W.P., Desmawati. 2020. Mealybugs (Hemiptera: Pseudococcidae) assosiated with dragon fruit in Indonesia. p. 29–34. In: Proceedings of the International Conference and the 10th Congress of the Entomological Society of Indonesia (ICCESI 2019): Advances in Biological Sciences Research. 6–9 October 2019, Bali, Indonesia. DOI: https://doi.org/10.2991/absr.k.200513.005

Sartiami D., Sihombing E.R., Balfas R. 2010. Identify mealybug species (Hemiptera: Pseudoccidae) on pepper plants (*Piper nigrum* LINN.). p. 217–227. In: Proceedings of National Seminar V: Empowering Insect Diversity to Improve Community Welfare. 18 Maret 2008, Bogor, Indonesia (in Indonesia)

Soorya V., Radhakrishnan V.V., Mohanan K.V. 2018. Association of aronomic characters in *Curcuma aeruginosa* Roxb. (pink and blue ginger). International Journal of Research and Analytical Reviews 5 (4): 142–146. Available on: http://ijrar.com/upload_issue/ijrar_issue_20542758.pdf

Wang Y.S., Zhou P., Tian H., Wan F.H., Zhang G.P. 2018. First record of the invasive pest *Pseudococcus jackbeardsleyi* (He-

miptera: Pseudococcidae) on the Chinese mainland and its rapid identification based on species-specific polymerase chain reaction. Journal of Economic Entomology 111 (5): 2120–2128. DOI: https://doi.org/10.1093/jee/toy223

Wieczorek K., Ball K., Durak R., Borowiak-Sobkowiak B. 2024. New alien and invasive bamboo aphid species of the genus *Takecallis* (Hemiptera: Aphididae) recorded in Poland – morphological and molecular identity. Journal of Plant Protection Research 64 (1): 69–76. DOI: https://doi.org/10.24425/jppr.2024.149155

Williams D.J. 2004. Mealybugs of Southern Asia. The Natural History Museum & Southdene, Kuala Lumpur, 896 pp.

Williams D.J., de Willink G.M.C. 1992. Mealybugs of Central and South America. CAB International, Wallingford, 635 pp.

Williams D.J., Watson G.W. 1988. Scale Insects of the Tropical South Pacific Region, Part 2. Mealybugs (Pseudococcidae). CAB International, Wallingford, United Kingdom, 260 pp.